

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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| Applicant's or agent's file reference<br>B650WOORD01 96705592                             | <b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |  |
| International application No.<br>PCT/EP00/00324   | International filing date (day/month/year)<br>18/01/2000  | Priority date (day/month/year)<br>19/01/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>A61K38/00 |   |  |
| Applicant<br>BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH                                     |   |  |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

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| Date of submission of the demand<br><br>16/08/2000  | Date of completion of this report<br><br>30.04.2001  |
| Name and mailing address of the international preliminary examining authority:<br> European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br><br>Ludwig, G<br><br>Telephone No. +49 89 2399 8698<br> |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00324

## I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

### Description, pages:

1-10 as originally filed

### Claims, No.:

1-17 as originally filed

### Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

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☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 13-15.

because:

☒ the said international application, or the said claims Nos. 13-15 relate to the following subject matter which does not require an international preliminary examination (*specify*):  
**see separate sheet**

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)

Yes: Claims 1-17

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|-------------------------------|------|--------|--|
|                               | No:  | Claims |  |
| Inventive step (IS)           | Yes: | Claims |  |
|                               | No:  | Claims | 1-17                                     |
| Industrial applicability (IA) | Yes: | Claims | 1-14, 16-17 (13-15 - cf. separate sheet) |
|                               | No:  | Claims |  |

2. Citations and explanations  
**see separate sheet**

**INTERNATIONAL PRELIMINARY  
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International application No. PCT/EP00/00324

- D1: US-A-5 840 527 (CORDELL BARBARA ET AL) 24 November 1998 (1998-11-24)
- D2: HARTSHORN KEVAN L ET AL: 'Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses.' JOURNAL OF CLINICAL INVESTIGATION, vol. 94, no. 1, 1994, pages 311-319, XP000939180 ISSN: 0021-9738
- D3: LEVINE ANN MARIE ET AL: 'Surfactant protein-A-deficient mice are susceptible to Pseudomonas aeruginosa infection.' AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY, vol. 19, no. 4, October 1998 (1998-10), pages 700-708, XP000939181 ISSN: 1044-1549
- D4: HARROD KEVIN S ET AL: 'SP-A enhances viral clearance and inhibits inflammation after pulmonary adenoviral infection.' AMERICAN JOURNAL OF PHYSIOLOGY, vol. 277, no. 3 PART 1, 1999, pages L580-L588, XP002148502 ISSN: 0002-9513 - **P-document**

*cf. the citations indicated in the International search report*

Item V:

1. In case that the priority of the application (not checked) is *not* valid, P-document D4 could be used against novelty/inventive step of the application.
2. Document D1 discloses the use of recombinant surface protein A (SP-A) for the treatment of respiratory distress syndrome (RDS) and related respiratory diseases such as pneumonia and bronchitis.  
A recombinant 32 and 10 kD alveolar surfactant protein were used alone and in combination (column 7, last paragraph, column 30, paragraph 2, fig.12, column 2, lines 52-54).
3. Document D2 discloses protection against influenza A virus by

surfactant protein D (SP-D), alone or in conjunction with surface protein A.

[Rat/human SP-D show a molecular weight on SDS-PAGE under reducing conditions of 43 kD; Human SP-A appears as a broad band of about 26-36 kD under these conditions - D2, page 311, right column, last paragraph, page 312, left column, paragraph 2 from the bottom].

4. Document D3 describes that surfactant protein-A-deficient mice are susceptible to infection by *Pseudomonas aeruginosa* which causes pulmonary infections.

It is concluded that SP-A plays an important role in the pathogenesis of mucoid *P.aeruginosa* infection in the lung in vivo by enhancing macrophage phagocytosis and clearance of bacteria and by modifying the inflammatory response.

5. The problem to be solved by invention is to provide a medicament for preventing or treating a pulmonary infection or inflammation.
6. Claims 1-3, 5-11, 13-14, and 16-17 are not regarded as inventive having regard to document D1 (alone or in combination with document D3).

Claims 4, 12, and 15 are not considered to be inventive in view of document D1 (alone or in conjunction with document D3) and D2.

7. For the assessment of the present claims 13-15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**INTERNATIONAL PRELIMINARY  
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International application No. PCT/EP00/00324

Item III:

8. Claims 13-15 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |           |  |
|--|-----------|--|
| <b>(51) International Patent Classification <sup>7</sup> :</b><br><b>A61K 38/00</b>  | <b>A2</b> | <b>(11) International Publication Number:</b> <b>WO 00/43026</b><br><b>(43) International Publication Date:</b> 27 July 2000 (27.07.00)  |
| <b>(21) International Application Number:</b> PCT/EP00/00324<br><b>(22) International Filing Date:</b> 18 January 2000 (18.01.00)<br><br><b>(30) Priority Data:</b><br>60/155,268 19 January 1999 (19.01.99) US<br><br><b>(71) Applicant (for all designated States except US):</b> BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH [DE/DE]; Byk-Gulden-Str. 2, D-78467 Konstanz (DE).<br><br><b>(72) Inventors (for all designated States except CA US):</b> MELCHERS, Klaus; Im Grund 13, D-78267 Aach (DE). SCHÄFER, Klaus, P.; Glockenbrunnenstr. 6, D-78465 Konstanz (DE).<br><br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> STEINHILBER, Wolfram [DE/DE]; Herzog-Erchanger-Str. 16, D-78333 Stockach (DE). WHITSETT, Jeffrey, A. [US/US]; 5565 Salem Road, Cincinnati, OH 45230 (US). LEVINE, Ann, Marie [US/US]; 4086 Georgetown Road, Cincinnati, OH 45236 (US). KORFHAGEN, Thomas, R. [US/US]; 1151 Hickorylake Drive, Cincinnati, OH 45233 (US). |           | <b>(74) Agent:</b> RUPP, Herbert; Byk Gulden Lomberg Chemische Fabrik GmbH, Byk-Gulden-Str. 2, D-78467 Konstanz (DE).<br><br><b>(81) Designated States:</b> AE, AL, AU, BA, BG, BR, CA, CN, CZ, EE, GE, HR, HU, ID, IL, IN, JP, KR, LT, LV, MK, MX, NO, NZ, PL, RO, SG, SI, SK, TR, UA, US, VN, YU, ZA, ZW, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).<br><br><b>Published</b><br><i>Without international search report and to be republished upon receipt of that report.</i> |
| <b>(54) Title:</b> RECOMBINANT SP-A FOR THE TREATMENT OR PREVENTION OF PULMONARY INFECTION AND INFLAMMATION<br><br><b>(57) Abstract</b><br><br>Recombinant surfactant protein A and medicament compositions based thereon are useful for the prevention or treatment of pulmonary infection and inflammation.  |           |  |



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## **Recombinant SP-A for the treatment or prevention of pulmonary infection and inflammation**

### **Technical field**

The present invention relates to the novel use of recombinant surfactant protein A for the production of a medicament for the treatment of pulmonary infection and inflammation.

### **Prior art**

Pulmonary surfactant plays an important role in maintaining the structural integrity of the alveoli by reducing surface tension. The surfactant consists mostly of a complex mixture of phospholipids and genetically distinct proteins referred to as surfactant protein A, B, C and D (also designated as SP-A, SP-B, SP-C and SP-D). It is synthesized by alveolar type II pneumocytes and secreted as tightly packed lamellar bodies into the alveoli (King, R. J.: Pulmonary Surfactant, J. Appl. Physiol. **1982**, 51, 1-8).

SP-A is hypothesized to play a role in protecting the lung from bacterial, viral, and fungal infections (Thiel, S., and Reid, K.: Structures and functions associated with the group of mammalian lectins containing collagen-like sequences, FEBS Lett. **1989**, 250, 78). In addition, in vitro, it has been shown that SP-A binds to various micro-organisms, acts as opsonin, enhances killing of micro-organisms by macrophages, down regulates pro-inflammatory cytokines such as TNF- $\alpha$  induced by LPS or microbial pathogens (reviewed in: Molecular Basis of Disease, Pulmonary surfactant. Ed: L.M.G. van Golde, Biochimica et Biophysica Acta, 1998, 1408, 77-364).

Recently, it was shown that mice lacking SP-A are susceptible to group B streptococcal infection (LeVine A. M. et al.: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection; The Journal of Immunology, **1997**, 4336-4340) and that exogenous proteinosis SP-A enhanced bacterial clearance in SP-A deficient mice (LeVine A. M. et al.: Surfactant protein-A (SP-A) binds group B streptococcus (GBS), enhancing phagocytosis and clearance from lungs of SP-A deficient mice; Am J. Respir. Crit. Care Med. 1998, Vol. 157, A 865). In addition, it was shown that baboons with bronchopulmonary dysplasia (BPD) and superimposed infection have decreased levels of SP-A present in the lungs (King, R. J., et al.: Surfactant protein-A deficiency in a primate model of pulmonary dysplasia, Am. J. Respir. Crit. Care Med. **1995**, 151(6), 1989-97 and Coalson, J. J.: Pathophysiologic, morphometric, and biochemical studies of the premature baboon with bronchopulmonary dysplasia, Am. Rev. Respir. Dis. **1992**, 145, 872-81). Furthermore, it was shown that SP-A is decreased in a number of diseases such as pneumonia, asthma, bronchiolitis, lung transplantations, cystic fibrosis, ARDS, smokers etc. as reviewed in M. Giese, Pulmonary surfactant in health and human lung diseases: state of the art. Eur. Respir. J. 1999, 13, 1455-1476. It was also shown, that SP-A inhibits allergen induced histamine release as well as the proliferation of lymphocytes in cells isolated from allergen exposed asthmatics. (Wang, J.Y. et al., Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release

in children with asthma. *Am. J. Respir. Crit. Care Med.*, 1998, 158, 510-518; Mandan, T. et al., Lung surfactants proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. *Clin. Exp. Immunol.*, 1997, 110, 241-249).

### Summary of the Invention

The subject invention has several distinct aspects. One aspect is the use of a component which is at least substantially the same as recombinant surfactant protein A (rSP-A) for treating or preventing a pulmonary infection or inflammation. Another aspect is a medicament composition for treating or preventing a pulmonary infection and inflammation, and which comprises an active component which is at least substantially the same as recombinant surfactant protein A. A further aspect is a method of compounding such a medicament composition. A still further aspect comprises the concurrent use of surfactant protein D (SP-D) with an active component which is at least substantially the same as recombinant surfactant protein A in treating or preventing a pulmonary infection and inflammation, in compounding a medicament composition and in the resulting medicament composition itself. An additional aspect of the invention is an article of manufacture, comprising packaging material and rSP-A or a compound which is substantially the same as rSP-A (in a container) within the packaging material, and the packaging material including a label or instructions which indicate usefulness for the treatment or prevention of inflammation or microbial infection.

### Brief Description of the Drawings

Figure 1 graphically illustrates that the clearance of GBS in the SP-A (-/-) mice was significantly enhanced at 6 and 24 hours when GBS was co-administered with 150 µg rSP-A.

Figure 2 graphically illustrates that GBS clearance by SP-A (-/-) mice is dose dependent and is increased by increased amount of rSP-A.

Figure 3 graphically illustrates the clearance of GBS in wild-type mice is significantly enhanced at 6 and 24 hours when GBS is co-administered with 150 µg rSP-A.

Figure 4 graphically illustrates that wild-type animals infected with GBS and treated 6 hours after infection with intratracheal rSP-A have increased clearance of GBS at 24 hours.

Figure 5 graphically illustrates that exogenous rSP-A reduces TNF-α content in lung homogenates from SP-A (-/-) mice challenged with GBS close to the level observed in SP-A (+/+) mice.

### Details

Surprisingly it has now been found that recombinant surfactant-associated protein A (rSP-A) can be used in the treatment or prevention of pulmonary infection and inflammation and is equivalent or superior to the use of surfactant-associated protein A (SP-A) obtained from natural sources, for example that isolated from lavage fluid from healthy individuals or proteinosis patients. This must be regarded as particularly surprising as SP-A isolated from human lung lavage consists of a homogenous population of a flower bouquet like

hexameric structure, each unit of which consists of three SP-A polypeptide chains ( $\alpha 1$ ), analogous to that described for the complement factor C1q. The fully assembled hexameric structure is thought to be essential for a functional molecule with respect to stimulating anti-microbial defense mechanisms or anti-inflammatory activity. In contrast to the naturally derived SP-A, surfactant-associated protein A produced by recombinant techniques consists of a variety of different oligomeric structures ranging from one single polypeptide chain ( $\alpha 1$ ) to the fully assembled octadecameric form ( $\gamma 6$ ) (Voss, T., et al.: Macromolecular organization of natural and recombinant lung surfactant protein SP 28-36; J. Mol. Biol. 1988, 201, 219-227; Voss et al.: Structural comparison of recombinant pulmonary surfactant protein SP-A derived from two human coding sequences: Implications for the chain composition of natural human SP-A, Am. J. Respir. Cell Mol. Biol., 1991, 4, 88-94).

In addition it was shown, that natural derived SP-A is glycosylated. However, although recombinant produced SP-A, depending on the system used for expression (mammalian, insect, or yeast cells), shows different glycosylation patterns, it shows anti-microbial or anti-inflammatory effects superior or equivalent to the natural SP-A.

As used herein microbial refers to bacterial, viral or fungal.

As used herein recombinant surfactant-associated protein A (hereinafter also referred to as rSP-A) refers to vertebrate, preferably mammalian, surfactant-associated protein A produced by recombinant techniques. Amino acid sequences and DNA sequences coding for mammalian surfactant-associated protein A are, for example, described in WO86/03408, WO88/05820 and USP 4,882,422. Recombinant surfactant-associated protein A further refers to derivatives of vertebrate, preferably mammalian, surfactant-associated protein A produced by recombinant techniques which differ from natural mammalian or other vertebrate surfactant-associated protein A by addition, deletion or substitution of amino acids as long as the proteins retain microbial clearance activity or anti-inflammatory activity. Such activity of recombinant surfactant-associated protein A can, for example, be determined in an assay according to the one herein-after described for group B streptococcus bacteria. In a preferred embodiment rSP-A refers to human surfactant-associated protein A produced by recombinant methods and having an amino acid sequence encoded by a DNA sequence contained in cDNA clones, pHS10-5, pHS10-4, PSAP-1A, PSAP-6A and a genomic clone pHS-15 or an allelic variation thereof. Two genes (designated as A1 and A2) coding for SP-A have been identified in the human genome (White, R. T. et al.: Nature 1985, 317, 361-363; Katyal, S. L. et al.: Am. J. Respir. Cell Mol. Biol., 1992, 6:446-452). The genomic clone pHS-15 coding for human SP-A has been described in WO86/03408, and clones containing cDNA sequences coding for SP-A have been described in WO88/05820 for pHS10-5 and pHS10-4, and by Floros et al., The Journal of Biological Chemistry, 1986, 261, 9029-33 and USP 4,882,422 for PSAP-1A and PSAP-6A). Recombinant SP-A may be obtained according to procedures known in the art. Methods for cloning and production of rSP-A are for example described in WO86/03408, WO88/05820, USP 4,659,805, USP 4,912,038, Voss, T., et al.: Macromolecular organization of natural and recombinant lung surfactant protein SP 28-36; J. Mol. Biol. 1988, 201, 219-227, and Voss et al.: Structural comparison of recombinant pulmonary surfactant protein SP-A derived from two human coding sequences: Implications for the chain composition of natural human SP-A, Am. J. Respir. Cell Mol. Biol., 1991, 4 88-94).

In the text and drawings *n* refers to the number of animals (mice), and *wt* refers to „wild-type“. As used in the claims, „substantially the same as“ includes a) derivatives of surfactant-associated protein A produced by recombinant techniques, but which differ from natural surfactant-associated protein A by addition, deletion or substitution of one or more amino acids, b) SP-A modifications which differ in type and/or degree of glycosylation, and c) recombinant fusion proteins consisting of the complete or portions of the SP-A fused with suitable proteins or parts thereof having anti-infective or anti-inflammatory activities, as long as the surfactant-associated proteins A retain microbial clearance activity or anti-inflammatory activity, as determined by assay.

Examples which may be mentioned in connection with deleted, truncated or mutated forms of rSP-A are the SP-A-glob variant in which the amino acids of the collagenous domain were deleted (aa 17-80) or other forms as described in Spissinger et al., Assembly of the surfactant protein SP-A. Eur. J. Biochem., 1991, 199, 65-71.

Exemplary proteins having anti-infective or anti-inflammatory activities which may be mentioned in connection with recombinant fusion proteins are proteins such as defensins, lysozymes, cytokines, chemokines and immunoglobulins. These proteins can be fused to either the C- or N-terminal end of SP-A.

In one embodiment of the invention recombinant SP-A obtainable by expression of a DNA sequence coding for SP-A in a suitable eucaryotic expression system is used for the manufacture of a medicament for the prevention or treatment of pulmonary infection and inflammation. Suitable expression systems are, for example, CHO-cells using suitable expression vectors. Suitable expression vectors are, for example: pMT(E) Apo containing the SV40 enhancer and the inducible human metallothionin promoter (Fritz et al., Proc. Natl. Acad. Sci. USA, 83:4114-4118), pRc/CMV for constitutive expression of the gene of interest (Invitrogen, Leek, Netherlands) or any other expression vector useful for mammalian cells containing homologous intron sequences (i.e., authentic genomic sequences from the gene of interest) or heterologous intron sequences. In this case it is further preferred to use a partial or complete genomic sequence coding for SP-A, for example a genomic sequence as described in WO86/03408 for the A1 gene yielding a higher expression rate and subsequently to higher order structures of SP-A (Voss et al.: Structural comparison of recombinant pulmonary surfactant protein SP-A derived from two human coding sequences: Implications for the chain composition of natural human SP-A, Am. J. Respir. Cell Mol. Biol., 1991, 4:88-94). This approach would also apply for the A2 gene described by Katyal, S. L. et al. (Am. J. Respir. Cell Mol. Biol., 1992, 6:446-452). Preferentially the expression of the genomic sequence coding for SP-A in a suitable expression system is carried out as described by Voss et. al. (Am. J. Respir. Cell Mol. Biol. 1991, 4, 88-94).

In addition, to express the cDNA sequences coding for SP-A (A1/A2) it is preferred to use either insect cells using the Baculovirus expression system (McCormack, F. et al., J. Biol. Chem., 1994, 269:5833-5841) or yeast, such as *Pichia pastoris*, in both cases with or without co-expression of the human prolyl 4-hydroxylase stabilizing the collagen helices by hydroxylating proline residues in the collagenous domain as demonstrated for the expression of collagen (Lamberg, Arja et al., J. Biol. Chem., 1996, 271:11988-

11995; Vuorela, A. et al., EMBO J., 1997, 16:6702-6712). For example rSP-A can be produced by cloning of the respective cDNAs into the EcoRI site of the Baculovirus expression vector pVL1392, subsequent generation of recombinant viruses and expression in SF21 cells using standard procedures. rSP-A may also be produced in yeast (for example *Pichia pastoris*) after cloning of the respective cDNAs into yeast expression vectors such as pPICZ A (Invitrogen, Leek, Netherlands) for the expression in *Pichia pastoris*.

In another embodiment of the invention recombinant SP-A obtainable by expression of a DNA sequence coding for SP-A in a suitable procaryotic expression system is used for the manufacture of a medicament for the prevention or treatment of pulmonary infection and inflammation.

In a further embodiment of the invention non-glycosylated rSP-A is used for the manufacture of a medicament for the prevention or treatment of pulmonary infection and inflammation.

A still further embodiment of the invention is an article of manufacture which comprises packaging material and a pharmaceutical agent within the packaging material wherein the pharmaceutical agent is at least substantially the same as rSP-A, and wherein the packaging material comprises a label or package insert which indicates that the pharmaceutical agent is useful for preventing or treating a pulmonary microbial infection or a pulmonary inflammation. The packaging material, label and package insert otherwise parallel or resemble what is generally regarded as standard packaging material, labels and package inserts for pharmaceuticals having related utilities.

## **Pharmacology**

### **METHODS**

#### **Animal Husbandry**

The murine SP-A gene locus was targeted by homologous recombination as previously described (Korfhagen; T.R. et al.: "Altered surfactant function and structure in SP-A gene targeted mice; PNAS, 1996, 93, 9594-9599). Lungs of SP-A (-/-) mice do not contain detectable SP-A mRNA or protein. To limit variability related to strain differences, 129 J wild type (+/+) and SP-A (-/-) mice of the same strain were studied. Animals were housed and studied under IACUC-approved protocols in the animal facility of the Children's Hospital Research Foundation, Cincinnati. Male and female mice of approximately 20 to 25 grams (35 to 42 days old) were used. (LeVine A. M. et al.: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection; The Journal of Immunology, 1997, 158, 4336-4340).

#### **Recombinant SP-A**

Recombinant SP-A was produced as described by Voss et al. (Am. J. Respir. Cell Mol. Biol. 1991, 4, 88-94).

### **Preparation of Bacteria**

A stock culture of group B streptococcus (GBS) was obtained from a clinical isolate from a newborn with systemic infection. Bacteria were suspended in sterile phosphate-buffered saline (PBS) containing 20 % glycerol and frozen in aliquots at  $-70^{\circ}\text{C}$ . Bacteria from the same passage were used to minimize variations in virulence related to culture conditions. Before each experiment, an aliquot was thawed and plated on tryptic soy-5 % defibrinated sheep blood agar then inoculated into 4 ml of Todd-Hewitt broth (Difco Laboratories, Detroit, MI) and grown for 14 to 16 hours at  $37^{\circ}\text{C}$  with continuous shaking. The broth was centrifuged, and the bacteria were washed in PBS at pH 7.2 and resuspended in 4 ml of the buffer. In order to facilitate studies, a growth curve was generated so the bacterial concentration could be determined spectrophotometrically, which was confirmed by quantitative culture of the intratracheal inoculum.

### **Intratracheal Inoculation**

Administration of GBS into the respiratory tract of the mice was performed by intratracheal inoculation of  $10^4$  or  $10^6$  cfu diluted in sterile normal saline (0.9 % NaCl). To deliver GBS in the presence of rSP-A, GBS was diluted in 0.9 % NaCl with 1 mM  $\text{Ca}^{2+}$  and appropriate amounts of rSP-A in a  $37^{\circ}\text{C}$  water bath for 30 minutes. Bacteria were delivered by intratracheal inoculation as previously described (LeVine A. M. et al.: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection; *The Journal of Immunology*, 1997, 4336-4340).

### **Bacterial Clearance**

Quantitative cultures of lung homogenates were performed 6 and 24 hours after inoculation of the animals with bacteria or bacteria together with SP-A, as previously described (LeVine A. M. et al.: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection; *The Journal of Immunology*, 1997, 4336-4340). Bacterial clearance from the lungs of SP-A (-/-) mice was determined after intratracheal inoculation with GBS together with varying doses of rSP-A ranging from 25  $\mu\text{g}$ , 50  $\mu\text{g}$ , 75  $\mu\text{g}$ , 100  $\mu\text{g}$  to 150  $\mu\text{g}$ . Wild-type animals were infected with GBS ( $10^6$  cfu) and treated with rSP-A (150  $\mu\text{g}$ ) at the time of infection or 6 hours after infection.

### **Cytokine Production**

Lung homogenates were centrifuged at  $1,200 \times g$  and the supernatants were stored at  $-20^{\circ}\text{C}$ . Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) levels were measured with ELISAs, using goat antimurine antibody (R&D Systems) directed against TNF- $\alpha$ . All plates were read on a microplate reader (Molecular Devices, Menlo Park, CA) and analyzed with the use of a computer-assisted analysis program (Softmax, Molecular Devices).

## Statistical Methods

Since the distribution of the variable cfu/gram of lung was not normally distributed, a natural log transformation was used for all analyses. Analyses of variance (ANOVA) was performed to assess differences between the groups. Individual scores for each time point were compared using the median scores non parametric test. Findings were considered statistically significant at probability levels  $<0.05$ .

## RESULTS

### Recombinant SP-A Increased Bacterial Clearance in SP-A (-/-) Mice

The clearance of GBS in the SP-A (-/-) mice was significantly enhanced at 6 and 24 hours when GBS was co-administered with 150  $\mu$ g rSP-A (Figure 1). Effects of rSP-A were comparable to that observed with SP-A isolated from proteinosis patients. GBS clearance by SP-A (-/-) mice was dose dependent and was increased by 50  $\mu$ g, 75  $\mu$ g, 100  $\mu$ g and 150  $\mu$ g of rSP-A (Figure 2).

### Recombinant SP-A Increased Bacterial Clearance in Wild-type Mice

The clearance of GBS in wild-type mice was significantly enhanced at 6 and 24 hours when GBS was co-administered with 150  $\mu$ g rSP-A (Figure 3). Wild-type animals infected with GBS and treated 6 hours after infection with intratracheal rSP-A (150  $\mu$ g) had increased clearance of GBS at 24 hours (Figure 4).

Pulmonary clearance of intratracheally administered GBS was reduced in SP-A (-/-) mice compared to wild-type mice. Co-administration of exogenous recombinant SP-A with the bacteria significantly improved bacterial clearance demonstrating an immediate reversible defect in the SP-A (-/-) mouse. Enhanced pulmonary clearance of GBS with rSP-A treatment was dose dependent. Wild-type mice were effectively treated with rSP-A with increased clearance of GBS from the lungs.

In addition, it could be shown that exogenous rSP-A reduces TNF- $\alpha$  content in lung homogenates from SP-A (-/-) mice challenged with GBS close to the level observed in SP-A (+/+) mice (Figure 5).

## Utility

On account of its microbial clearance and anti-inflammatory properties rSP-A is useful for the manufacture of medicaments for the prevention or treatment of pulmonary infection and inflammation. As used herein pulmonary infection refers to microbial pneumonias caused, for example, by viruses like *Respiratory Syncytial Virus*, *Adenovirus*, *Herpes simplex*, *Influenza A* and others or bacteria like, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Group B Streptococci*, *Enterobacter* or *Streptococcus pneumoniae* and others, as well as fungi like *Aspergillus fumigatus*, *Pneumocystis carinii*, *Candida albicans* and others.



Furthermore pulmonary infection also refers to cases of reduced immunity for example the immunoparalytic phase occurring during sepsis and to immunodeficiency syndromes, whether congenital, spontaneously acquired, or iatrogenic. They are characterized by unusual susceptibility to infection and not infrequently to autoimmune disease and lymphoreticular malignancies. Patients with defects in humoral immunity have recurrent or chronic sinopulmonary infection, meningitis, and bacteremia, most commonly caused by pyogenic bacteria, such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Staphylococci*. These and other pyogenic organisms also cause frequent infections in individuals who have either neutropenia or a deficiency of the pivotal third component of complement (C3).

In connection with the present invention pulmonary inflammation refers, e.g., to bronchopulmonary dysplasia (BPD), and rSP-A therefore is also useful for the manufacture of medicaments for the prevention or treatment of bronchopulmonary dysplasia (BPD) or other disorders of SP-A deficiency. In particular BPD caused by artificial ventilation of premature babies may be mentioned in connection with the present invention. Additionally, inflammation also refers to pulmonary inflammation caused by artificial ventilation or cases of release of cytokines into the lung not primarily due to bacteria, viruses or fungi. Inflammation includes also diseases like asthma, CF (cystic fibrosis) and COPD (chronic obstructive pulmonary disease). It also includes acute lung injury up to the worst stages known as ARDS. In addition, inflammation also refers to inflammation induced by allergens.

The present invention also refers to the treatment of bacterial pulmonary infections with rSP-A or suitable forms thereof in combination or addition to antibiotics in the way that the infection induced inflammation is cured.

The invention furthermore relates to a method for the treatment of mammals, including humans, who are suffering from one of the above-mentioned illnesses. The method is characterized in that a therapeutically active and pharmacologically tolerable amount of rSP-A is administered to the mammal in need thereof.

In connection with the novel use of rSP-A according to the invention medicaments are prepared by procedures familiar to those skilled in the art. To do this rSP-A is either employed as such or preferably in combination with suitable pharmaceutical auxiliaries, e.g., as suspensions, solutions or in powder form, the rSP-A content advantageously being from 0.1 to 90 % (wt/wt), preferably 0.1 to 15 % (wt/wt). The rSP-A can be administered either alone or with auxiliaries. The auxiliaries which are suitable for the desired pharmaceutical formulations are familiar to the person skilled in the art on account of his expert knowledge. Pharmaceutical formulations which can be used according to the present invention and containing rSP-A in combination with synthetic or natural lipids are, for example, disclosed in USP 4,659,805. Surprisingly, and in contrast to pharmaceutical formulations comprising rSP-A disclosed in the state of the art, lipid free formulations comprising rSP-A as active ingredient can also be used for the treatment of pulmonary infection and inflammation. A further aspect the invention thus relates to lipid free medicaments comprising rSP-A.

In particular such lipid free medicaments comprising rSP-A contain as auxiliary from 0.01 to 0.1 % of a calcium salt, preferably calcium chloride. An exemplary liquid, lipid free medicament can be manufactured

by dissolving 100 to 1,000 mg of rSP-A and 11.1 mg of calcium chloride in 100 ml of a sterile 0.9 % aqueous sodium chloride solution.

In a preferred embodiment the medicaments are made available in liquid form for intratracheal or intrabronchial administration by instillation or nebulization or in powder form for administration by inhalation.

In connection with the novel use of rSP-A according to the invention medicaments are administered, for example, 2 to 3 times daily for from 1 to 7 days. For example medicaments comprising 100µg/kg to 10mg/kg (of body weight) of rSP-A are administered by inhalation or intratracheally or intrabronchially.

In a further aspect of the invention rSP-A is administered in combination with surfactant proteins SP-B, SP-C and/or SP-D or their modified derivatives for the treatment or prevention of pulmonary infection and inflammation. In particular the co-administration of rSP-A with SP-D is preferred in connection with the invention. The amino acid sequences of said surfactant proteins, their isolation or preparation by genetic engineering are known (e.g. from WO86/03408, EP-A-0 251 449, WO89/04326, WO87/06943, WO88/03170, EP-A-0 368 823, EP-A-0 348 967, WO91/18015, WO95/32992, EP-A-0 593 094, WO92/22315 and Crouch et al.: Recombinant Pulmonary Surfactant Protein D, The Journal of Histological Chemistry, 1994, 269, 15808-15813 and references cited therein).

#### Description of figures

**Figure 1:** Enhanced clearance of GBS from the lung in SP-A (-/-) mice,  $10^4$  cfu GBS were inoculated with or without 150 µg of rSP-A, and colony counts were performed after 6 and 24 hours as previously described. SP-A (-/-) without rSP-A (solid bars) and with rSP-A (hatched bars). Data are Means  $\pm$  SEM (standard error of means) values.

**Figure 2:** Enhanced clearance of GBS by exogenous rSP-A is dose dependent. SP-A (-/-) mice were inoculated with  $10^4$  cfu GBS in the presence of either 0 (N.T.), 25, 50, 75, 100 or 150 µg of rSP-A, and colony counts were performed 6 hours after intratracheal instillation as previously described. Data are Means  $\pm$  SEM values.

**Figure 3:** Enhanced clearance of GBS from the lung in SP-A (+/+) mice (wt).  $10^6$  cfu GBS were inoculated with or without 150 µg of rSP-A and colony counts were performed 6 and 24 hours after intratracheal instillation as previously described. SP-A (+/+) without rSP-A (hatched bars) and with rSP-A (solid bars). Data are Means  $\pm$  SEM values.

**Figure 4:** Rescue of GBS infection in SP-A(wt) mice. SP-A (+/+) mice were intratracheally inoculated with  $10^6$  cfu GBS in the absence of exogenous rSP-A. 6 hours after infection, 150 µg of rSP-A were intratracheally administered. 24 hours after rSP-A administration colony counts in lungs were performed. Colony counts were dramatically reduced in lungs from animals treated with rSP-A (solid bars) compared to untreated animals (hatched bars). Data are Means  $\pm$  SEM values.

**Figure 5:** rSP-A reduces TNF- $\alpha$  content in lung homogenates from SP-A (-/-) mice challenged with GBS. SP-A (-/-) and SP-A (+/+) mice were intratracheally inoculated with  $10^4$  cfu GBS, in the absence or presence of exogenous 150  $\mu$ g rSP-A. 6 hours after infection lungs were removed, homogenized, and the level of TNF- $\alpha$  was measured as previously described. SP-A (-/-) untreated (solid bar), SP-A (-/-) treated (cross hatched bar), SP-A (+/+) mice untreated (dotted bar). Data are expressed in pg/ml and represent means  $\pm$  SEM values with n = 6 mice per group.

The invention and its advantages are readily understood from the preceding description. Various changes may be made in the processes and compositions without departing from the spirit and scope of the invention or sacrificing its material advantages, the processes and products hereinbefore described being merely illustrative of preferred embodiments of the invention.

**WHAT IS CLAIMED IS:**

1. In a method for compounding a medicament composition for preventing or treating a pulmonary infection or inflammation and comprising suitable carrier and a pharmaceutically-acceptable active component, the improvement wherein the active component is at least substantially the same as recombinant surfactant protein A (rSP-A).
2. A method of claim 1 wherein rSP-A is recombinant SP-A which is the same as that obtainable by expression of a genomic sequence coding for SP-A in a suitable expression system.
3. A method of claim 1 wherein rSP-A is recombinant SP-A which is the same as that obtainable by expression of a cDNA coding for SP-A in a suitable expression system.
4. A method of claim 1 wherein the medicament composition further comprises surfactant protein D (SP-D).
5. A lipid-free medicament composition which is useful for treating or preventing a pulmonary infection or inflammation and which comprises an effective amount of pharmaceutically-acceptable active component and suitable carrier therefor, wherein the active component is at least substantially the same as rSP-A.
6. A medicament composition of claim 5 for treating or preventing a pulmonary infection.
7. A medicament composition of claim 6 wherein the pulmonary infection is bacterial, viral or fungal pneumonia.
8. A medicament composition of claim 5 for treating or preventing a pulmonary inflammation.
9. A medicament composition of claim 8 wherein the pulmonary inflammation is bronchopulmonary dysplasia.
10. A medicament composition of claim 5 wherein the rSP-A is at least substantially the same as recombinant SP-A obtained by expression of a genomic sequence coding for SP-A in a suitable expression system.
11. A medicament composition of claim 5 wherein the rSP-A is at least substantially the same as recombinant SP-A obtained by expression of a cDNA coding for SP-A in a suitable expression system.

12. A medicament composition of claim 5 wherein the active component further comprises surfactant protein D (SP-D).
13. In a method for preventing or treating a pulmonary infection or inflammation in a subject prone to or afflicted with such condition, which method comprises administering an effective amount of pharmaceutically-acceptable active component to the subject, the improvement wherein the active component comprises an essential ingredient which is at least substantially the same as recombinant surfactant protein A (rSP-A).
14. A method of claim 13 wherein the essential ingredient is rSP-A.
15. A method of claim 13 wherein the active component further comprises surfactant protein D (SP-D).
16. An article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, wherein the pharmaceutical composition comprises an active agent which is at least substantially the same as recombinant surfactant protein A, and wherein the packaging material comprises a label or package insert which indicates that the active agent is useful for preventing or treating a pulmonary microbial infection or inflammation.
17. An article of manufacture of claim 16 wherein the active agent is rSP-A.

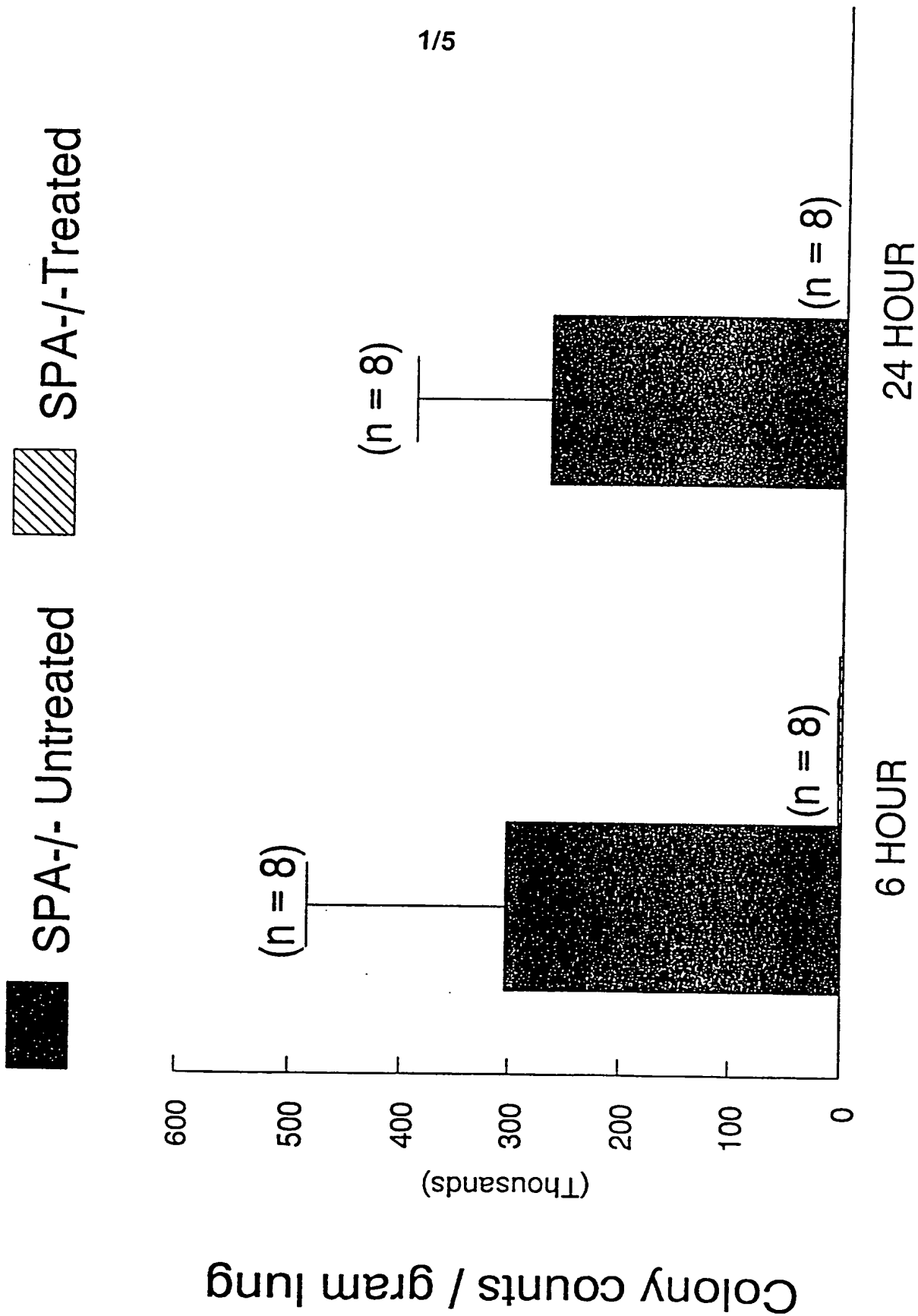


Figure 1

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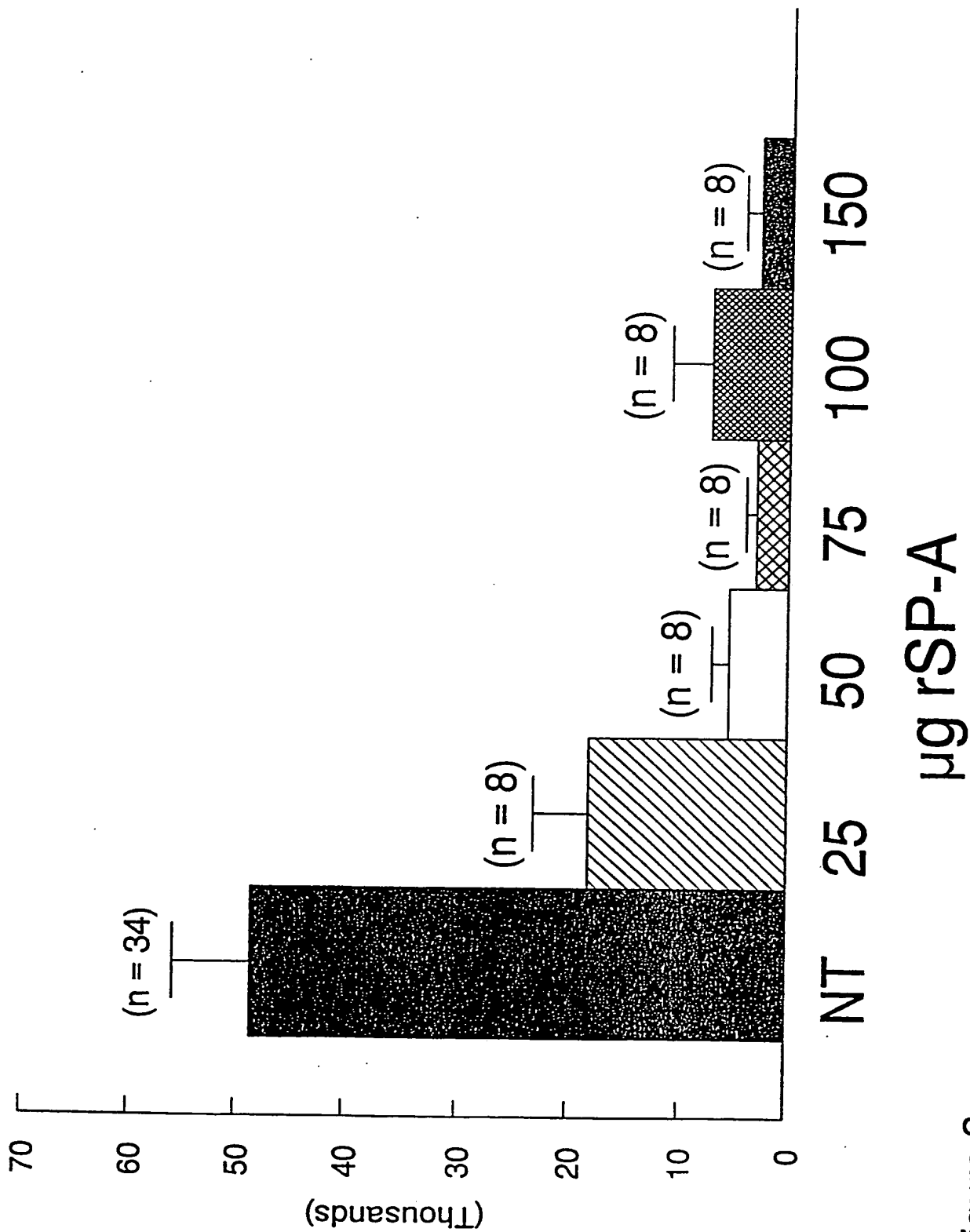


Figure 2

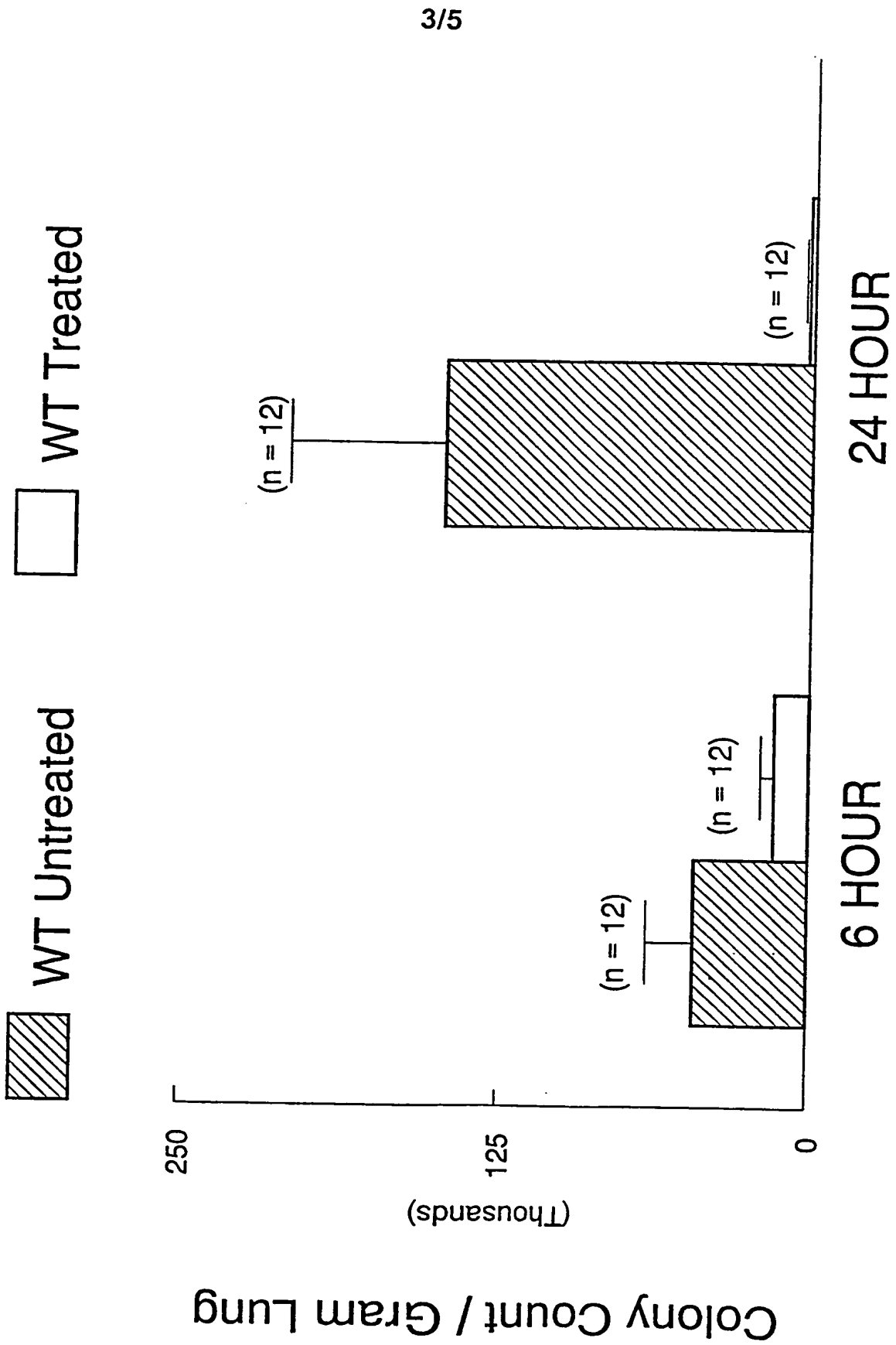


Figure 3



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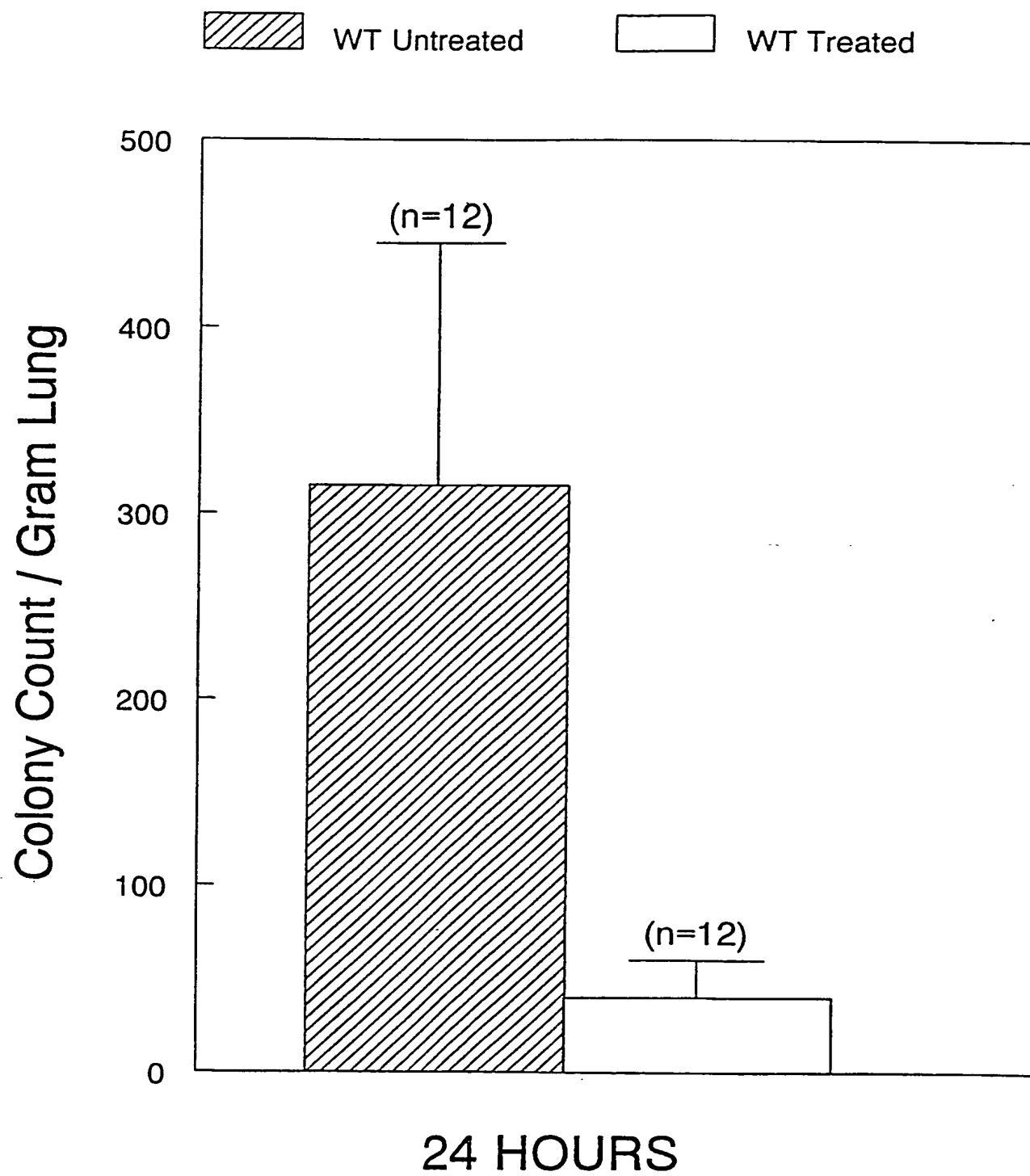


Figure 4

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TNF

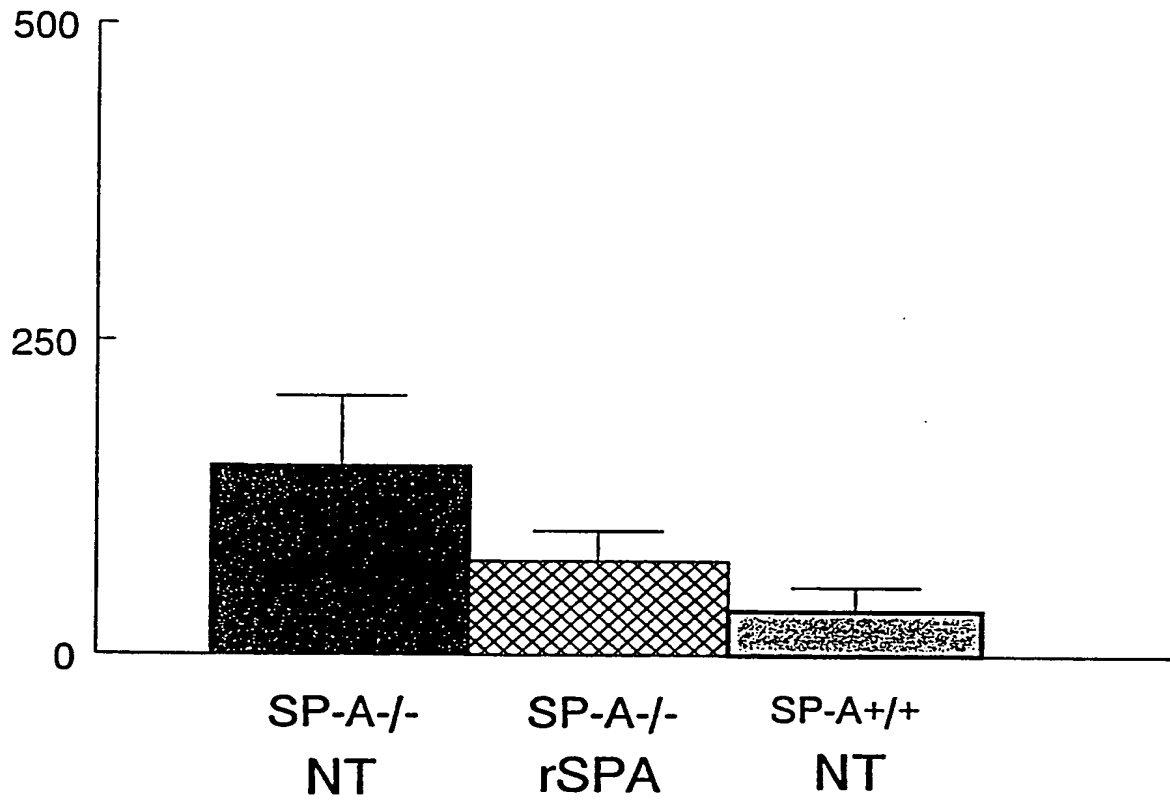


Figure 5

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

|  |   |  |
|--|---|--|
| Applicant's or agent's file reference<br><b>B650WOORD01</b>      | <b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |  |
| International application No.<br><b>PCT/EP 00/ 00324</b>         | International filing date (day/month/year)<br><b>18/01/2000</b>   | (Earliest) Priority Date (day/month/year)<br><b>19/01/1999</b> |
| Applicant<br><br><b>BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH</b> |   |  |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
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- ☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of Invention is lacking** (see Box II).

**4. With regard to the title,**

- ☒ the text is approved as submitted by the applicant.
- ☐ the text has been established by this Authority to read as follows:

**5. With regard to the abstract,**

- ☒ the text is approved as submitted by the applicant.
- ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

**6. The figure of the drawings to be published with the abstract is Figure No.**

- ☐ as suggested by the applicant.
- ☐ because the applicant failed to suggest a figure.
- ☐ because this figure better characterizes the invention.
- ☒ None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 00/00324

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 A61K38/17 A61P11/00 A61P31/04 A61P31/10 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
|------------|--|-------------------------|
| X          | US 5 840 527 A (CORDELL BARBARA ET AL)<br>24 November 1998 (1998-11-24)  | 1-3,<br>5-11, 13,<br>14 |
| Y          | column 2, line 39 - line 55; figure 1<br><br>column 6, line 3 - line 5<br>column 6, line 30 - line 45<br>column 7, line 68 - line 66<br>column 8, line 35 - line 63<br><br>-----<br>-/-- | 4-7, 12,<br>15          |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"&" document member of the same patent family

Date of the actual completion of the international search

27 September 2000

Date of mailing of the international search report

10/10/2000

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Noë, V

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/00324

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| X          | HARTSHORN KEVAN L ET AL: "Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses."<br>JOURNAL OF CLINICAL INVESTIGATION,<br>vol. 94, no. 1, 1994, pages 311-319,<br>XP000939180<br>ISSN: 0021-9738  | 1                     |
| Y          | abstract<br><br>page 316, column 1, paragraph 3 -page 317,<br>column 1, paragraph 2<br>page 317, column 2, last line<br>page 318, column 1, paragraph 2<br>table 2   | 4-7, 12,<br>15        |
| A          | ---<br>LEVINE ANN MARIE ET AL: "Surfactant protein-A-deficient mice are susceptible to Pseudomonas aeruginosa infection."<br>AMERICAN JOURNAL OF RESPIRATORY CELL AND MOLECULAR BIOLOGY,<br>vol. 19, no. 4, October 1998 (1998-10),<br>pages 700-708, XP000939181<br>ISSN: 1044-1549<br>the whole document |                       |
| P, X       | ---<br>HARROD KEVIN S ET AL: "SP-A enhances viral clearance and inhibits inflammation after pulmonary adenoviral infection."<br>AMERICAN JOURNAL OF PHYSIOLOGY,<br>vol. 277, no. 3 PART 1, 1999, pages<br>L580-L588, XP002148502<br>ISSN: 0002-9513<br>the whole document<br>-----                         | 1, 6-8                |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/00324

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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Date of mailing  
(day/month/year) 30.04.2001

Applicant's or agent's file reference  
B650WOORD01 96705592

**IMPORTANT NOTIFICATION**

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18/01/2000

Priority date (day/month/year)  
19/01/1999

Applicant  
BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

**4. REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office  
D-80298 Munich  
Tel. +49 89 2399 - 0 Tx: 523656 epmu d

Authorized officer

Hundt, D



PCT/IPEA/416

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C.20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

|  |   |
|--|---|
| Date of mailing (day/month/year)<br>28 September 2000 (28.09.00)         |   |
| International application No.<br>PCT/EP00/00324                          | Applicant's or agent's file reference<br>B650WOORD01 96705592 |
| International filing date (day/month/year)<br>18 January 2000 (18.01.00) | Priority date (day/month/year)<br>19 January 1999 (19.01.99)  |
| Applicant<br>STEINHILBER, Wolfram et al                                  |   |

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

16 August 2000 (16.08.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

|   |   |
|---|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No.: (41-22) 740.14.35 | Authorized officer<br>F. Baechler<br>Telephone No.: (41-22) 338.83.38 |
|---|---|



PCT

NOTIFICATION CONCERNING  
SUBMISSION OR TRANSMITTAL  
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

RUPP, Herbert  
Byk Gulden Lomberg Chemische  
Fabrik GmbH  
Byk-Gulden Str. 2  
D-78467 Konstanz  
ALLEMAGNE

Date of mailing (day/month/year)

04 May 2000 (04.05.00)

Applicant's or agent's file reference

B650WOORD01 96705592

International application No:

PCT/EP00/00324

International filing date (day/month/year)

18 January 2000 (18.01.00)

International publication date (day/month/year)

Not yet published

Priority date (day/month/year)

19 January 1999 (19.01.99)

Applicant

BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH et al

## IMPORTANT NOTIFICATION

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An asterisk(\*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The letters "NR" appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority datePriority application No.Country or regional Office  
or PCT receiving OfficeDate of receipt  
of priority document

19 Janu 1999 (19.01.99)

60/155,268

US

12 Apr 2000 (12.04.00)

The International Bureau of WIPO  
34, chemin des C. lombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Céline Faust



Telephone No. (41-22) 338.83.38

002284346

PCT/IB/304

SRX

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

RUPP, Herbert  
Byk Gulden Lomberg Chemische  
Fabrik GmbH  
Byk-Gulden-Str. 2  
D-78467 Konstanz  
ALLEMAGNEEINGANG  
RECEIVED

04 Aug. 2000

Gewerblicher  
Rechtsschutz

|   |  |  |  |
|---|--|--|--|
| Date of mailing (day/month/year)<br>27 July 2000 (27.07.00)   |  | IMPORTANT NOTICE   |  |
| Applicant's or agent's file reference<br>B650WOORD01/96705592 |  |  |  |
| International application No.<br>PCT/EP00/00324               | International filing date (day/month/year)<br>18 January 2000 (18.01.00) | Priority date (day/month/year)<br>19 January 1999 (19.01.99) |  |
| Applicant<br>BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH et al   |  |  |  |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

AU,JP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AL,BA,BG,BR,CA,CN,CZ,EA,EE,EP,GE,HR,HU,ID,IL,IN,LT,LV,MK,MX,NO,NZ,PL,RO,SG,SI,  
SK,TR,UA,VN,YU,ZA,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 27 July 2000 (27.07.00) under No. WO 00/43026

## REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

## REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

|  |   |
|--|---|
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No. (41-22) 740 14 35 | Authorized officer<br>J. Zahra<br>Telephone No. (41-22) 338 83 38 |
|--|---|

PCT/IB/308

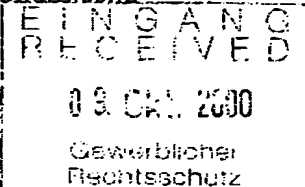
From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED  
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

RUPP, Herbert  
Byk Gulden Lomborg Chemische  
Fabrik GmbH  
Byk-Gulden-Str. 2  
D-78467 Konstanz  
ALLEMAGNE

|  |  |  |  |
|--|--|--|--|
| Date of mailing (day/month/year)<br>28 September 2000 (28.09.00) |  | IMPORTANT INFORMATION  |  |
| Applicant's or agent's file reference<br>B650WOORD01/96705592    |  |  |  |
| International application No.<br>PCT/EP00/00324                  | International filing date (day/month/year)<br>18 January 2000 (18.01.00) | Priority date (day/month/year)<br>19 January 1999 (19.01.99) |  |
| Applicant<br>BYK GULDEN LOMBERG CHEMISCHE FABRIK GMBH et al      |  |  |  |

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

EP : AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE  
National : AU, BG, CA, CN, CZ, IL, JP, KR, NO, NZ, PL, RO, SK, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

EA : AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
National : AE, AL, BA, BR, EE, GE, HR, HU, ID, IN, LT, LV, MK, MX, SG, SI, TR, UA, VN, YU, ZA,  
ZW

3. The applicant is reminded that he must enter the "national phase" before the expiration of 30 months from the priority date before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

The entry into the European regional phase is postponed until 31 months from the priority date for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Authorized officer:

F. Baechler

PCT IB/332

PCT

## REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference  
(if desired) (12 characters maximum) B650WOORD01 96705592

## Box No. I TITLE OF INVENTION

Recombinant SP-A for the treatment or prevention of pulmonary infection and inflammation

## Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

Byk Gulden  
Lomberg Chemische Fabrik GmbH  
Byk-Gulden-Str. 2  
D-78467 Konstanz  
DE

☐ This person is also inventor.

Telephone No.

07531/84 5338

Facsimile No.

07531/84 5321

Teleprinter No.

State (that is, country) of nationality:

DE

State (that is, country) of residence:

DE

This person is applicant  
for the purposes of:

☐

all designated States

☒

all designated States except the United States of America

☐

the United States of America only

☐

the States indicated in the Supplemental Box

## Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

STEINHILBER, Wolfram  
Herzog-Erchanger-Str. 16  
D-78333 Stockach  
DE

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

DE

State (that is, country) of residence:

DE

This person is applicant  
for the purposes of:

☐

all designated States

☐

all designated States except the United States of America

☒

the United States of America only

☐

the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

## Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:

☒

agent

☐

common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Herbert Rupp  
c/o Byk Gulden  
Lomberg Chemische Fabrik GmbH  
Byk-Gulden-Str. 2  
D-78467 Konstanz  
DE

Telephone No.

07531/84 5314

Facsimile No.

07531/84 5321

Teleprinter No.

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent

PCT/RO/101

## Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

*If none of the following sub-boxes is used, this sheet should not be included in the request.*

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

MELCHERS, Klaus  
Im Grund 13  
D-78267 Aach  
DE

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☒ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

SCHÄFER, Klaus P.  
Glockenbrunnenstr. 6  
D-78465 Konstanz  
DE

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☒ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

WHITSETT, Jeffrey A.  
5565 Salem Road  
Cincinnati, OH 45230  
U.S.A.

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

U.S.A.

State (that is, country) of residence:

U.S.A.

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

LEVINE, Ann Marie  
4086 Georgetown Road  
Cincinnati, OH 45236  
U.S.A.

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

U.S.A.

State (that is, country) of residence:

U.S.A.

This person is applicant for the purposes of:

- ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet

## Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

KORFHAGEN, Thomas R.  
1151 Hickorylake Drive  
Cincinnati, OH 45233  
U.S.A.

This person is:

☐ applicant only☒ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:  
U.S.A.

State (that is, country) of residence:  
U.S.A.

This person is applicant  
for the purposes of:

☐ all designated States☐ all designated States except the United States of America☒ the United States of America only☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant  
for the purposes of:

☐ all designated States☐ all designated States except the United States of America☐ the United States of America only☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant  
for the purposes of:

☐ all designated States☐ all designated States except the United States of America☐ the United States of America only☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

☐ applicant only☐ applicant and inventor☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant  
for the purposes of:

☐ all designated States☐ all designated States except the United States of America☐ the United States of America only☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box No. III:

MELCHERS, Klaus and SCHÄFER, Klaus P. are inventors for all designated states except the United States of America and except Canada.

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

## Regional Patent

- ☐ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line) .....

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- |   |  |
|---|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates       | <input type="checkbox"/> LR Liberia  |
| <input checked="" type="checkbox"/> AL Albania                    | <input type="checkbox"/> LS Lesotho  |
| <input type="checkbox"/> AM Armenia                               | <input checked="" type="checkbox"/> LT Lithuania                                 |
| <input type="checkbox"/> AT Austria                               | <input type="checkbox"/> LU Luxembourg   |
| <input checked="" type="checkbox"/> AU Australia                  | <input checked="" type="checkbox"/> LV Latvia                                    |
| <input type="checkbox"/> AZ Azerbaijan                            | <input type="checkbox"/> MA Morocco  |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina     | <input type="checkbox"/> MD Republic of Moldova                                  |
| <input type="checkbox"/> BB Barbados                              | <input type="checkbox"/> MG Madagascar   |
| <input checked="" type="checkbox"/> BG Bulgaria                   | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BR Brazil                     | <input type="checkbox"/> MN Mongolia   |
| <input type="checkbox"/> BY Belarus                               | <input type="checkbox"/> MW Malawi   |
| <input checked="" type="checkbox"/> CA Canada                     | <input checked="" type="checkbox"/> MX Mexico                                    |
| <input type="checkbox"/> CH and LI Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> NO Norway                                    |
| <input checked="" type="checkbox"/> CN China                      | <input checked="" type="checkbox"/> NZ New Zealand                               |
| <input type="checkbox"/> CR Costa Rica                            | <input checked="" type="checkbox"/> PL Poland                                    |
| <input type="checkbox"/> CU Cuba                                  | <input type="checkbox"/> PT Portugal   |
| <input checked="" type="checkbox"/> CZ Czech Republic             | <input checked="" type="checkbox"/> RO Romania                                   |
| <input type="checkbox"/> DE Germany                               | <input type="checkbox"/> RU Russian Federation                                   |
| <input type="checkbox"/> DK Denmark                               | <input type="checkbox"/> SD Sudan  |
| <input type="checkbox"/> DM Dominica                              | <input type="checkbox"/> SE Sweden   |
| <input checked="" type="checkbox"/> EE Estonia                    | <input checked="" type="checkbox"/> SG Singapore                                 |
| <input type="checkbox"/> ES Spain                                 | <input checked="" type="checkbox"/> SI Slovenia                                  |
| <input type="checkbox"/> FI Finland                               | <input checked="" type="checkbox"/> SK Slovakia                                  |
| <input type="checkbox"/> GB United Kingdom                        | <input type="checkbox"/> SL Sierra Leone   |
| <input type="checkbox"/> GD Grenada                               | <input type="checkbox"/> TJ Tajikistan   |
| <input checked="" type="checkbox"/> GE Georgia                    | <input type="checkbox"/> TM Turkmenistan   |
| <input type="checkbox"/> GH Ghana                                 | <input checked="" type="checkbox"/> TR Turkey                                    |
| <input type="checkbox"/> GM Gambia                                | <input type="checkbox"/> TT Trinidad and Tobago                                  |
| <input checked="" type="checkbox"/> HR Croatia                    | <input type="checkbox"/> TZ United Republic of Tanzania                          |
| <input checked="" type="checkbox"/> HU Hungary                    | <input checked="" type="checkbox"/> UA Ukraine                                   |
| <input checked="" type="checkbox"/> ID Indonesia                  | <input type="checkbox"/> UG Uganda   |
| <input checked="" type="checkbox"/> IL Israel                     | <input checked="" type="checkbox"/> US United States of America                  |
| <input checked="" type="checkbox"/> IN India                      | <input type="checkbox"/> UZ Uzbekistan   |
| <input type="checkbox"/> IS Iceland                               | <input checked="" type="checkbox"/> VN Viet Nam                                  |
| <input checked="" type="checkbox"/> JP Japan                      | <input checked="" type="checkbox"/> YU Yugoslavia                                |
| <input type="checkbox"/> KE Kenya                                 | <input checked="" type="checkbox"/> ZA South Africa                              |
| <input type="checkbox"/> KG Kyrgyzstan                            | <input checked="" type="checkbox"/> ZW Zimbabwe                                  |
| <input type="checkbox"/> KP Democratic People's Republic of Korea |  |
| <input checked="" type="checkbox"/> KR Republic of Korea          |  |
| <input type="checkbox"/> KZ Kazakhstan                            |  |
| <input type="checkbox"/> LC Saint Lucia                           |  |
| <input type="checkbox"/> LK Sri Lanka                             |  |

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

**Precautionary Designation Statement:** In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation (including fees) must reach the receiving Office within the 15-month time limit)



| Filing date of earlier application (day/month/year) | Number of earlier application | Where earlier application is: |                                       |   |
|---|-------------------------------|-------------------------------|---------------------------------------|---|
|   |                               | national application: country | regional application: regional Office | international application: receiving Office |
| item (1)<br>(19.01.1999)<br>19 January 1999         | 60/155,268                    | US                            |                                       |   |
| item (2)  |                               |                               |                                       |   |
| item (3)  |                               |                               |                                       |   |

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s):

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

#### Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA /

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year)

Number

Country (or regional Office)

#### Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request : 7  
description (excluding sequence listing part) : 10  
claims : 2  
abstract : 1  
drawings : 5  
sequence listing part of description :  
Total number of sheets : 25

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☒ priority document(s) identified in Box No. VI as item(s): (1)
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other (specify):

Figure of the drawings which should accompany the abstract:

Language of filing of the international application:

English

#### Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

.....  
Dr. Herbert Rupp  
European Patent Attorney

Byk Gulden  
Lomborg Chemische Fabrik GmbH

i.V. Dr. Robert Wild i.V. Dr. Bernd Kratzer

| For receiving Office use only   |  |
|---|--|
| 1. Date of actual receipt of the purported international application:   | 2. Drawings:   |
| 3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: | <input type="checkbox"/> received:   |
| 4. Date of timely receipt of the required corrections under PCT Article 11(2):  | <input type="checkbox"/> not received:   |
| 5. International Searching Authority (if two or more are competent): ISA /  | 6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid. |

Date of receipt of the record copy by the International Bureau:

For International Bureau use only

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box No. IX:

01-24-00

Date

Dr. Wolfram STEINHILBER

Date

Jeffrey A. WHITSETT, M.D.

Date

Ann Marie LEVINE, M.D.

Date

Thomas R. KORFHAGEN, M.D., Ph.D.

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box No. IX:

Date

Dr. Wolfram STEINHILBER

Date

Jeffrey A. WHITSETT, M.D.

Date

Ann Marie LEVINE, M.D.

Date

Thomas R. KORFHAGEN, M.D., Ph.D.